REMARKS

Claims 1, 3 to 7, 9 to 15 and 17 to 25 are currently pending. Claims 17 to 20 are withdrawn from consideration. Claims 23 to 25 are new. Support for this new claim can be found, e.g., in original claim 1, page 3, lines 23 to 25 (see replacement paragraph introduced per preliminary amendment of April 10, 2007), but in particular, page 6, line 29 to page 7, line 26.

On pages 2 to 4, the Office rejected the claims 1, 3 to 7, and 9 to 15 under 35 USC §112, first paragraph, as not being enabled.

The Office expressed the opinion that, while the specification is enabling for alternations of a plasmin cleavage site by substitution of amino acids corresponding to positions 110 and/or 111 of VEGF165 in a VEGF molecule, the specification does not reasonably provide enablement for alternations of positions corresponding of positions corresponding to positions 109 or 112.

For the sole purpose of furthering the prosecution in this case, applicants have amended claim 1. The claim now calls for least one amino acid at positions 110 or 111 of the native vascular endothelial growth factor to be replaced by proline or to be deleted. Claim 3 was amended accordingly.

Applicants submit that the claim as amended are enabled for, among others, the reasoning provided by the Office on pages 3 and 4 of the Office Action.

On pages 4 to 6, the Office rejects claims 1, 3 to 7, 9 to 15 and 21 to 22 as obvious under 35 USC §103(a) over Keyt at al. (J. Biochem. 271(13): 7788-7795 (1996)) and Lauer et al. (J. Invest. Dermatol. 115:12-18 (2000)) in view of Markert et al. (Protein Engineer. 14(10):791-796 (2001)) and U.S. Pat. No. 5,219,739 to Tischer et al.

The Office acknowledges that neither Keyt nor Lauer teach a VEGF variant wherein amino acid positions 110 and/or 111 have been mutated or deleted.

However, the Office expressed the opinion that it was well known that plasmin cleaves at dibasic amino acid pairs and that therefore there is a reasonable expectation that, if either the

amino acids at positions 110 or 111 (or both amino acids) are substituted such that they are no longer a dibasic pair, then the plasmin will no longer be able to cleave the protein.

The Office also expresses the opinion that Markert et al. teach site directed mutagenesis to provide proteolytic resistance to enzymatic degradation and that Tischer et al. teach the amino acid and nucleic acid sequences for $VEGF_{121}$ and $VEGF_{165}$ (see previous Office Action).

The Office came to the conclusion that it would have been obvious to one of ordinary skill in the art at the time the invention was made to make a VEGF molecule that was resistant to plasmin cleavage using the methods of Markert et al. and the starting materials of Tisher et al.

Applicants note that there is no clear consensus sequence for the cleavage site of plasmin. However, as many serine proteases, plasmin preferably cleaves after basic amino acids such as arginine (Arg). This also applies for cleavage site 110 Arg/111 Ala of the VEGF 165 molecule (see Keyt). The influence of any amino acid following after the basic amino acid, in the example provided, alanine (Ala), was not known. Alanine (Ala) has, in contrast to the basic amino acid arginine (Arg), a non-polar aliphatic side chain. The inventors surprisingly discovered that a replacement of an amino acid (e.g., alanine (Ala)) that followed after the basic amino acid with, e.g., proline (Pro) leads to plasmin stability while biological activity is maintained. Proline (Pro) has, as alanine (Ala), a non-polar aliphatic site chain, though a more complex one. In fact, as detailed on page 4, lines 9 to 17, proline is a cyclic alpha-imino acid and has been known as a helix breaker. Surprisingly, the replacement of one non-basic amino acid having a non-polar aliphatic side chain, e.g., alanine (Ala), with in this case, proline (Pro), resulted in a VEGF that is not only stable towards the protease plasmin, but still has an activity corresponding to that of the wild type protein.

The Office's attention is in particular directed at claims 3, 4, 5 and 7, 10, 21 and 22 and new claims 23 to 25. With regard to new claim 25, applicants notes that lysine (Lys) is, like the arginine (Arg) is replaces, a basic amino acid.

Applicants respectfully submit that, with respect to claims 3, 4, 5 and 7, 10, 21 and 22 and new claims 23 to 25, which all encompass changes in amino acid 111, involving, e.g., the non-basic amino acid alanine (Ala), no *prima facie* case of obviousness has been established (MPEP §2142). In particular and as outlined above, no reasoning has been provided why a person of skill in the art would as modified position 111 of the VEGF.

With regard to claims 1 and all other claims directly or indirectly dependent thereon, applicants also submit that no *prima facie* case of obviousness has been established (MPEP §2142). The Office relied in the rejection on combining prior art elements according to known methods to yield predictable results MPEP §2143). However, no specific motivation is provided for the substitutions/deletions as claimed. Considering the specific substitutions claims (substitution with proline/deletion), applicants respectfully submit that the results would have not been predictable. Applicants respectfully submit that, as disclosed in the specification, e.g., proline is known to be helix breaker. Surprisingly, it has been found that the replacement of an amino acid at position 111 results in a protease resistant VEGF variant that still has an activity corresponding to the wild-type protein. In addition, mutations at position 110 also result in higher stability of the VEGF (see, e.g., Figure 2C and corresponding description in the paragraph bridging page 11 and 12). Accordingly, applicants submit that no *prima facie* case was established with regard to the specified claims.

Applicants have shown above, that claims 1, 3 to 7 and 9 to 15 and 21 to 25 are enabled and are non-obvious over the cited art.

An early notice of allowance is therefore respectfully requested.

A two months extension of time is submitted herewith. The Commissioner is authorized to charge any fee deficiencies and overpayments to deposit account number 50-3135.

Respectfully submitted,

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